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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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10/574,085

04/16/2007

Koji Sode

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EXAMINER

SAIDHA, TEKCHAND

ART UNIT

PAPER NUMBER

1652

NOTIFICATION DATE

DELIVERY MODE

01/28/2011

ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No. 10/574,085	Applicant(s) SODE, KOJI	
	Examiner Tekchand Saidha	Art Unit 1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 29 November 2010.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1,4-7 and 9-15 is/are pending in the application.
- 4a) Of the above claim(s) 9-15 is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1 and 4-7 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|---|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____ |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

Final Rejection

1. Amendment and arguments filed 11/29/2010 are acknowledged. Claims 1 & 4-7 drawn to a fusion protein of pyrroloquinoline quinone glucose dehydrogenase (PQQGDH) and a cytochrome (SEQ ID NO: 2) are under consideration.

2. Applicant's arguments filed with the amendment cited above have been fully considered but they are not deemed to be persuasive. The reasons are discussed following the rejection(s).

3. Any objection or rejection of record not expressly repeated in this Office Action has been overcome by Applicant's response and withdrawn.

4. Claims withdrawn:

Claims 9-15 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

5. **35 U.S.C. § 112, first paragraph (Written Description)**

Claims 1 & 4-7 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

Claims 1 & 4-7 are broadly drawn to a fusion protein of pyrroloquinoline quinone glucose dehydrogenase (PQQGDH) and a cytochrome (claim 1), the claimed genus. Dependent claims 4-7 drawn to a fusion protein of claim 1, wherein the cytochrome is cytochrome c or cytochrome B562 (claim 4); or wherein the cytochrome is derived from a quinoxinoprotein which is a protein having both PQQ and a heme in one molecule (claim 5); or wherein the cytochrome is derived from a quinoxinoprotein alcohol dehydrogenase (claim 6); or wherein the cytochrome is derived from quinoxinoprotein ethanol dehydrogenase from *Comamonas testosteroni* (claim 7).

The specification, however, only provides description of a single species of DNA (SEQ ID NO: 1) encoding the fusion protein of SEQ ID NO: 2 for direct electron transfer-type glucose sensor for measuring blood glucose level.

The specification does not contain any disclosure or description of the structure and function of all fusion protein constructs comprising pyrroloquinoline quinone glucose dehydrogenase (PQQGDH) and a cytochrome from any source or from specific source with no defined structure or a fusion protein construct wherein the protein comprising an amino acid sequence, in which one or more amino acid residues have been deleted, substituted or added in the amino acid sequence of SEQ ID NO: 2 and having a glucose dehydrogenase activity and an electron transfer ability. The single species disclosed (SEQ ID NO: 2) is not representative of the genus claimed. According to MPEP 2163, to satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. See, e.g., *Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1319, 66 USPQ2d 1429, 1438 (Fed.Cir. 2003); *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116.

The scope of each genus includes many members of fusion construct with widely differing structural, chemical, and physical characteristics. Furthermore, each genus is highly variable because a significant number of structural differences between genus members exist. The specification does not describe and define any structural features and amino acid sequences commonly possessed by each genus. There is no art-recognized correlation between any structure of a fusion protein and the sequence of SEQ ID NO: 2 and wherein such fusion proteins have the desired glucose dehydrogenase activity and an electron transfer ability. Those of ordinary skill in the art would not be able to identify without further testing what specific DNA sequences that can be prepared and would encode the desired fusion protein having glucose dehydrogenase activity and an electron transfer ability.

The genus of fusion protein may be obtained with the aid of a computer by a skilled artisan. However, there is no teaching regarding how the sequences obtained from different sources can be fused recombinantly that can be varied and fused and still result in a DNA encoding a protein having glucose dehydrogenase activity and an electron transfer ability.

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An important consideration is that structure is not necessarily a reliable indicator of function. The instant specification provides no disclosure relating similarity or identity of structure to conservation of function. General knowledge in the art provides guidance to modification of some amino acids that are tolerated without losing a protein's tertiary structure. An important consideration is that structure is not necessarily a reliable indicator of function. In this example, there is no disclosure relating similarity of structure to conservation of function. General knowledge in the art included the knowledge that some amino acid variations are tolerated without losing a protein's tertiary structure. The results of amino acid substitutions have been studied so extensively that amino acids are grouped in so-called "exchange groups" of similar properties because substituting within the exchange group is expected to conserve the overall structure. For example, the expectation from replacing leucine with isoleucine would be that the protein would likely retain its tertiary structure. On the other hand, when non-exchange group members are substituted, e.g., proline for tryptophan, the expectation would be that the substitution would not likely conserve the protein's tertiary structure. Given what is known in the art about the likely outcome of substitutions on structure, those in the art would have likely expected the applicant to have been in possession of a genus of proteins having a tertiary structure similar to SEQ ID NO: 2 although the claim is not so limited. However, conservation of structure is not necessarily a surrogate for conservation of function. In this case, there is no disclosed correlation between structure and function. There is no disclosure of the active site amino acid residues responsible for the catalytic activity. While general knowledge in the art may have allowed one of skill in the art to identify other proteins expected to have the same or similar tertiary structure, in this case there is no general knowledge in the art about similar proteins to SEQ ID NO: 2 to suggest that general similarity of structure confers the activity. Accordingly, one of skill in the art would not accept the disclosure of SEQ ID NO: 2 (or the encoding DNA of SEQ ID NO: 1) as representative of other proteins having glucose dehydrogenase activity and an electron transfer ability. The specification, taken with the

pre-existing knowledge in the art of amino acid substitution and the genetic code, fails to satisfy the written description requirement of 35 U.S.C. 112, first paragraph.

Applicants' arguments:

Applicants argue that in order "To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. See, e.g., *Moba, B.V.v. Diamond Automation, Inc.*, 325 F.3d 1306, 1319, 66 USPQ2d 1429, 1438 (Fed. Cir. 2003); *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116.

The descriptive text in the specification needed to meet the written description requirement, however, varies with the nature and scope of the invention at issue, and with the scientific and technologic knowledge already in existence. The law must be applied to each invention that enters the patent process, for each patented advance is novel in relation to the state of the science. Since the law is applied to each invention in view of the state of relevant knowledge, its application will vary with differences in the state of knowledge in the field and differences in the predictability of the science, see *Fallmer v. Inglis*, 448 F.3d 1357, 79 USPQ2d 1001 (Fed. Cir. 2006).

Example 11B of the Guidelines, entitled "Art-Recognized Structure-Function Correlation Present" exemplifies a genus of nucleic acids that complies with the written description requirement. The claimed genus described in Example 11B encompasses nucleic acids that encode a polypeptide of a specified sequence and those that encode any polypeptide having at least 85% structural identity to the specified sequence, wherein the polypeptide, additionally, has activity Y. According to the Guidelines, the genus described in Example 11B complies with the written description requirement, inter alia, because the specification identifies two domains responsible for activity Y, i.e., a binding domain and a catalytic domain. Accordingly, there is information about which nucleic acids can vary in the corresponding nucleotide sequence in the claimed genus of nucleic acids and still encode a polypeptide having activity Y. In view of the foregoing, the exemplified claim complies with the written description requirement.

Applicants arguments are considered but not found to be persuasive because the amended claim 1 do justify the arguments presented nor is there any correlation between Example 11B and the instant claims as no homology or structural identity of the specified sequence of SEQ ID NO: 2 is recited in the claims. The instant claim 1 is drawn to a fusion protein of pyrroloquinoline quinone glucose dehydrogenase (PQQGDH) and a cytochrome, wherein the cytochrome has been fused to the C- terminal side of PQQGDH, and wherein the PQQGDH is either (a) or (b):

(a) a protein comprising an amino acid sequence represented by SEQ ID NO: 2;

(b) a protein comprising an amino acid sequence in which one or more amino acid residues have been deleted, substituted or added in the amino acid sequence (a) and having a glucose dehydrogenase activity and an electron transfer ability.

There is no limit to the extent of modification that is sought claim 1(b), and this is not equivalent to the description of mutants of *Acinetobacter ealcoacetieus* PQQGDH that were available in the art before or shortly after the priority date of the present invention, as per Appendix and Exhibits A1-A3, B1-B10, and C1-C16, as argued by the Applicants. The mutants described in the enclosed documents comprise specific amino acid deletions, substitutions, or additions and are not randomly generated. Further, SEQ ID NO: 2 consists of 591 amino acid residues.

While enzymatic assays are well known in the art, and the skilled artisan can produce variants of the polypeptide of SEQ ID NO: 2 having the recited structural characteristics using well-known and widely used techniques in the art, the amount of experimentation required is not routine due to the fact that the number of species encompassed by the claims is extremely large. Guo et al. (PNAS 101(25):9205-9210, 2004) teach that the percentage of random single substitution mutations which inactivate a protein for the protein 3-methyladenine DNA glycosylase is 34% (x factor) and that this number appears to be consistent with other studies in other proteins as well (Abstract). Guo et al. further shows in Table 1 that the percentage of active mutants for multiple mutants appears to be exponentially related to this by the simple formula $(.66)^x \times 100\%$ where x is the number of

mutations introduced and 0.66 is the probability of a protein to remain active after one amino acid change ($0.66 = 1 - 0.34$). If one were to apply this estimate to (for example) 95% sequence identity to SEQ ID NO: 2 (591 amino acids; 30 mismatches = 0.05×591), only $(.66)^{30} \times 100\%$ or $1.05 \times 10^{-4}\%$ of random mutants having 95% sequence identity to SEQ ID NO: 2 would be active. As indicated above, 95% sequence identity to SEQ ID NO: 2 allows for 30 amino acid changes. Therefore, to find a single active mutant within random mutants having 95% sequence identity to SEQ ID NO: 2, one of skill in the art would have to screen over several million mutants ($100 / 1.05 \times 10^{-4}\%$). This is only when 5% of the sequence is modified and does not include specific amino acid deletions, ~~substitutions~~, or additions. The level of unpredictability can only be imagined if the entire sequence of SEQ ID NO: 2 is modified as is encompassed by the language of claim 1(b).

Description to such is sequence is lacking. The rejection is maintained for the all the above reasons including the ones listed in body of the rejection and for which no specific response has been received.

6. Claim Rejections - 35 USC § 112, first paragraph (Enablement)

Claims 1 & 4-7 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a fusion protein of SEQ ID NO: 2 for direct electron transfer-type glucose sensor for measuring blood glucose level, does not reasonably provide enablement for all fusion protein constructs comprising pyrroloquinoline quinone glucose dehydrogenase (PQQGDH) and a cytochrome from any source or from specific source with no defined structure or a fusion protein construct wherein the protein comprising an amino acid sequence, in which one or more amino acid residues have been deleted, substituted or added in the amino acid sequence of SEQ ID NO: 2 and having a glucose dehydrogenase activity and an electron transfer ability. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of fusion constructs comprising

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individual elements of fusion constructs various modified and broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to the nucleotide sequence of SEQ ID NO: 1 and encoded amino acid sequence of fusion protein of SEQ ID NO: 2, having a glucose dehydrogenase activity and an electron transfer ability. While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass obtaining any fusion protein comprising PQQGDH and a cytochrome from any source or wherein the fusion protein is variously modified by modifying the protein/DNA sequence to any extent by insertion, deletion or substitution, and encoding or retaining the glucose dehydrogenase activity and electron transfer ability, because the specification does not establish: (A) regions of the protein structure which may be modified without effecting fusion protein activity; (B) the general tolerance of fusion protein to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any fusion protein residues with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including fusion protein constructs with an enormous number of nucleic acid/amino acid modifications of the sequence(s) of SEQ ID NO: 1/2 [as a result of modifying the DNA]. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of the numerous fusion protein constructs having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir, 1988).

Applicants' arguments:

Applicants argue that "The Federal Circuit has repeatedly held that "the specification must teach those skilled in the art how to make and use the full scope of the claimed invention without 'undue experimentation'." In re Wright, 999 F.2d 1557, 1561, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). Nevertheless, not everything necessary to practice the invention need be disclosed. In fact, what is well-known is best omitted. In re Buchner, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991). All that is necessary is that one skilled in the art be able to practice the claimed invention, given the level of knowledge and skill in the art, see also MPEP 2164.08.

As noted above, at the time of the invention an ordinary artisan would have recognized from art known at the time of filing which amino acids in SEQ ID NO: 2 could have been altered without a concomitant loss of glucose dehydrogenase activity and electron transfer ability. Accordingly, given the level of knowledge and skill in the art, an ordinary artisan would have been able to practice the invention without undue experimentation. In view of the foregoing, withdrawal of the rejection is respectfully requested.

Applicants' arguments are considered but not found to be persuasive because "There is no limit to the extent of modification that is sought in claim 1(b), and this is not

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equivalent to the enablement of selected mutants of *Acinetobacter ealcoacetieus* PQQGDH that were available in the art before or shortly after the priority date of the present invention, as per Appendix and Exhibits A1-A3, B1-B10, and C1-C16, as argued by the Applicants. The mutants described in the enclosed documents comprise specific amino acid deletions, substitutions, or additions and are enabling for selective modifications.

While enzymatic assays are well known in the art, and the skilled artisan can produce variants of the polypeptide of SEQ ID NO: 2 having the recited structural characteristics using well-known and widely used techniques in the art, the amount of experimentation required is not routine due to the fact that the number of species encompassed by the claims is extremely large. Guo et al. (PNAS 101(25):9205-9210, 2004) teach that the percentage of random single substitution mutations which inactivate a protein for the protein 3-methyladenine DNA glycosylase is 34% (x factor) and that this number appears to be consistent with other studies in other proteins as well (Abstract). Guo et al. further shows in Table 1 that the percentage of active mutants for multiple mutants appears to be exponentially related to this by the simple formula $(.66)^x \times 100\%$ where x is the number of mutations introduced and 0.66 is the probability of a protein to remain active after one amino acid change ($0.66 = 1 - 0.34$). If one were to apply this estimate to (for example) 95% sequence identity to SEQ ID NO: 2 (591 amino acids; 30 mismatches = 0.05×591), only $(.66)^{30} \times 100\%$ or $1.05 \times 10^{-4}\%$ of random mutants having 95% sequence identity to SEQ ID NO: 2 would be active. As indicated above, 95% sequence identity to SEQ ID NO: 2 allows for 30 amino acid changes. Therefore, to find a single active mutant within random mutants having 95% sequence identity to SEQ ID NO: 2, one of skill in the art would have to screen over several million mutants ($100 / 1.05 \times 10^{-4}\%$). This is only when 5% of the sequence is modified and does not include specific amino acid deletions, substitutions, or additions. The level of unpredictability can only be imagined if the entire sequence of SEQ ID NO: 2 is modified as is encompassed by the language of claim 1(b).

The rejection is therefore maintained for all the reasons of record.

7. **Claim Rejections - 35 USC § 103**

Rejection of Claims 1 & 4-7 as being rejected under 35 U.S.C. 103(a) as being unpatentable over Koji Hayade [WO 02/073181 A (9/19/2002)] **or** Sode, Koji [PrePub document No. 20050067278, 3/13/2001], Koji Hayade [JP 2002-125689 A (5/8/2002), Document 2], and Oubrie et al. [JBC 277(5): 3727-3732 (2002)] is being withdrawn in view of persuasive arguments by the Applicants.

8. During a telephone interview on 1/18/2011 with Applicants representative Linda T. Parker, the following claim amendment was proposed.

Claim 1 (rewrite as) A fusion protein of pyrroloquinoline quinone glucose dehydrogenase (PQQGDH) and a cytochrome, wherein the cytochrome has been fused to the C-terminal side of PQQGDH, and wherein the PQQGDH is either (a) or (b):

(a) a protein comprising an amino acid sequence represented by SEQ ID NO: 2;

(b) a protein comprising an amino acid sequence in which one or more amino acid residue has been deleted, substituted or added in the amino acid sequence (a) and having a glucose dehydrogenase activity and an electron transfer ability.

However, the discussion did not result in an agreement being reached hence this Office Action.

9. No claim is allowed.

10. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

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10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Tekchand Saidha whose telephone number is (571) 272 0940. The examiner can normally be reached between 8.30 am - 5.00 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert B. Mondesi can be reached on (571) 272 0956. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Tekchand Saidha/

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January 24, 2011